

Amendments to the Claims:

This listing of claims will replace all prior versions and listings of claims in the application:

Listing of Claims:

1. (Currently amended) A method for the detection of cytosine methylation in DNA samples, ~~characterized in that~~ comprising the following steps ~~are conducted~~:

chemically treating a genomic DNA sample which comprises unmethylated DNA to be investigated, which is the target DNA, and methylated DNA, which is the background DNA, is chemically treated such that all unmethylated cytosine bases are converted to uracil, while the 5-methylcytosine bases remain unchanged;

amplifying the chemically treated DNA sample is amplified with the use of at least 2 primer oligonucleotides as well as a polymerase and a nucleotide mixture, the composition of which leads to a preferred amplification of the target DNA over the background DNA wherein said nucleotide mixture contains one of (i) 2'-deoxyguanosine triphosphate (dGTP), 2'-deoxyadenosine triphosphate (dATP), 2'-deoxythymidine triphosphate (dTTP), wherein dTTP may be alternatively replaced with 2'-deoxyuridine triphosphate (dUTP), and 2'-deoxycytidine triphosphate (dCTP), wherein the initial concentration of dCTP is at most half as much as the average initial concentration of the other three nucleotides in said nucleotide mixture, and (ii) 2'-deoxycytidine triphosphate (dCTP), 2'-deoxyadenosine triphosphate (dATP), 2'-deoxythymidine triphosphate (dTTP), wherein dTTP may be alternatively replaced with 2'-deoxyuridine triphosphate (dUTP), and 2'-deoxyguanosine triphosphate (dGTP), wherein the initial concentration of dGTP is at most half as much as the average initial concentration of the other

three nucleotides in said nucleotide mixture; and

concluding the methylation state in the target DNA ~~is concluded~~ from the presence of an amplificate or its quantity.

2. (Canceled)

3. (Canceled)

4. (Canceled)

5. (Canceled)

6. (Canceled)

7. (Canceled)

8. (Canceled)

9. (Currently amended) The method according to claim 1, ~~further characterized in that~~
wherein the amplifying is performed in presence of additional terminating dideoxynucleotides are
~~additionally used in the amplification.~~

10. (Currently amended) The method according to claim 1, ~~further characterized in that~~
wherein the amplifying is performed as PCR amplification and wherein the a denaturing
temperature lies of below 90 °C ~~in the PCR amplification~~ is selected.

11. (Currently amended) The method according to claim 1, ~~further characterized in that~~
wherein the sample DNA is obtained from serum, plasma, urine, sputum or other body fluids of
an individual.

12. (Currently amended) The method according to claim 1, ~~further characterized in that~~
wherein the ~~chemical treatment~~ chemically treating is conducted with a bisulfite, disulfite, or

hydrogen sulfite containing solution.

13. (Currently amended) The method according to claim 12, ~~further characterized in that~~ wherein the ~~chemical treatment~~ ~~chemically treating~~ is conducted after embedding the DNA in agarose.

14. (Currently amended) The method according to claim 12, ~~further characterized in that~~ wherein in the chemical treatment, a reagent that denatures the DNA duplex and/or a radical scavenger is present.

15. (Currently amended) The method according to claim 1, ~~further characterized in that~~ wherein the ~~amplification~~ ~~amplifying~~ is conducted in the presence of at least one ~~other~~ additional oligonucleotide or a PNA oligomer, which binds to a nucleic acid comprising a 5'-CG-3' dinucleotide ~~or a 5'-tG-3' dinucleotide or a 5'-Ca-3' dinucleotide~~, whereby the ~~other~~ additional oligonucleotide or PNA oligomer preferably binds to the background DNA and adversely affects its amplification and ~~wherein "t" represents a thymine at a position which correlates with an unmethylated cytosine prior to bisulfite treatment and "a" correlates with such a thymine position.~~

16. (Currently amended) The method according to claim 15, ~~further characterized in that~~ wherein this binding site of the ~~other~~ additional oligonucleotide or PNA oligomer overlaps with the binding sites of the primers on the background DNA, and said ~~other~~ additional oligonucleotide or PNA oligomer thus impedes the binding of at least one primer oligonucleotide to the background DNA.

17. (Currently amended) The method according to one of claims 15 or 16, ~~further~~

~~characterized in that~~ wherein at least two ~~other~~ additional oligonucleotides or PNA oligomers are used, whereby their binding sites again each overlap with the binding site of a primer to the background DNA and said ~~other~~ additional oligonucleotides and/or PNA oligomers thus impede the binding of both primer oligonucleotides to the background DNA.

18. (Currently amended) The method according to claim 15, ~~further characterized in that~~ wherein these ~~other~~ additional oligonucleotides and/or PNA oligomers are present in at least five times the concentration of the primer oligonucleotides.

19. (Currently amended) The method according to claim ~~1~~ 15, ~~further characterized in that~~ wherein the polymerase used has no 5'-3' exonuclease activity.

20. (Currently amended) The method according to claim ~~1~~ 15, ~~further characterized in that~~ wherein the ~~other~~ additional oligonucleotides are modified at the 5'-end and thus cannot be significantly degraded by a polymerase with 5'-3' exonuclease activity.

21. (Currently amended) The method according to claim 1, ~~further characterized in that~~ wherein the primers in the amplification distinguish between target DNA and background DNA.

22. (Currently amended) The method according to claim ~~21~~ 1, ~~further characterized in that~~ wherein the background DNA is methylated, while the target DNA is unmethylated, each at positions at which at least one primer for the amplification binds, whereby the one or more primers preferably bind to the target DNA after the chemical treatment.

23. (Currently amended) The method according to claim 1, ~~further characterized in that~~ wherein the step of amplifying the chemically treated DNA sample is performed in presence of additionally at least one reporter oligonucleotide is used in the amplification whose fluorescence

properties change as a consequence of the amplification.

24. (Currently amended) The method according to claim ~~21~~ 23, ~~further characterized in that~~ wherein the step of amplifying the chemically treated DNA sample is performing by conducting a Taqman assay or a LightCycler assay or an assay with the use of Molecular Beacons ~~is conducted~~ to conclude upon the methylation state at the last step of the method.

25. (Currently amended) The method according to one of claims 23 or 24, ~~further characterized in that~~ wherein the reporter oligonucleotide bears at least one fluorescent label.

26. (Currently amended) The method according to one of claims ~~18~~ 23 to ~~22~~ 25, ~~further characterized in that~~ wherein the reporter oligonucleotide or the reporter oligonucleotides indicates or indicate the amplification either by an increase or a decrease in the fluorescence.

27. (Currently amended) The method according to claim 26, ~~further characterized in that~~ wherein the increase or decrease in fluorescence is used directly for the analysis and a conclusion on the methylation state of the DNA to be analyzed is made from the fluorescent signal.

28. (Currently amended) The method according to claim 1, ~~further characterized in that~~ wherein the background DNA is present in 100X the concentration in comparison to the target DNA.

29. (Currently amended) The method according to claim 1, ~~further characterized in that~~ wherein the background DNA is present in 1000X the concentration in comparison to the target DNA.

30. (Canceled)

31. (Currently amended) The method according to claim 1, ~~further characterized in that~~

wherein the amplicates themselves bear a detectable label for the detection.

32. (Currently amended) The method according to claim 31, ~~further characterized in that~~
wherein the labels are fluorescent labels.

33. (Currently amended) The method according to claim 31, ~~further characterized in that~~
wherein the labels are radionuclides.

34. (Currently amended) The method according to claim 31, ~~further characterized in that~~
wherein the labels are removable mass labels which are detected in a mass spectrometer.

35. (Currently amended) The method according to claim 1, ~~further characterized in that~~
wherein during amplification, one of the primers is bound to a solid phase.

36. (Currently amended) The method according to claim 1, ~~further characterized in that~~
wherein all the amplicates are detected in the mass spectrometer and are thus clearly
characterized by their mass.

37. (Canceled)

38. (Canceled)

39. (Canceled)

40. (Currently amended) A kit consisting of a reagent containing a bisulfite, primers for
the amplification and a nucleotide mixture ~~according to claim 2~~ one of (i) 2'-deoxyguanosine
triphosphate (dGTP), 2'-deoxyadenosine triphosphate (dATP), 2'-deoxythymidine triphosphate
(dTTP) or 2'-deoxyuridine triphosphate (dUTP), and 2'-deoxycytidine triphosphate (dCTP),
wherein the initial concentration of dCTP is at most half as much as the average initial
concentration of the other three nucleotides in said nucleotide mixture, and (ii) 2'-deoxycytidine

triphosphate (dCTP), 2'-deoxyadenosine triphosphate (dATP), 2'-deoxythymidine triphosphate (dTTP) or 2'-deoxyuridine triphosphate (dUTP), and 2'-deoxyguanosine triphosphate (dGTP), wherein the initial concentration of dGTP is at most half as much as the average initial concentration of the other three nucleotides in said nucleotide mixture.